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Simulating Burial Settings: Laboratory-Scale Forensic Bioreactor

VeeAnder Sheldonia Mealing

Clemson University, vmealn@g.clemson.edu

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SIMULATING BURIAL SETTINGS: LABORATORY-SCALE FORENSIC
BIOREACTOR

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Bioengineering

by
VeeAnder Sheldonia Mealing
August 2016

Accepted by:
Melinda Harman, Ph.D., Committee Chair
Caye Drapcho, Ph.D.
Elena Mikhailova, Ph.D.

ABSTRACT

Laboratory-scale forensic bioreactors can be beneficial for simulating and monitoring of burial settings by providing controlled environmental parameters (e.g. temperature, moisture, and others) applicable to a wide range of environments. The objectives of this study were to design and build forensic bioreactor, define parameters that are relevant to burial settings and suitable for laboratory simulation in the bioreactor, and verify the performance of the bioreactor. The laboratory-scale forensic bioreactor consisted of housing with individual soil chambers, temperature sensors with signal controls, soil moisture sensors, and a computer with software. The forensic bioreactor was capable of simulating burial settings. Two soil types with different soil pH levels and soil moisture within a udic moisture regime ($>10\%$ VWC) were placed in the bioreactor along with sensors and signal controls to maintain a thermic temperature regime ($15 - 22^{\circ}\text{C}$). The temperature parameters remained stable within the thermic temperature regime ($15 - 22^{\circ}\text{C}$) and toggled between 18.8°C (minimum temperature) and 20.2°C (maximum temperature). The soil moisture parameters declined slowly throughout the test period but remained within a udic moisture regime, averaging 22.0%, 17.6%, and 23.2% in the control, Ultisol, and Mollisol, respectively. The laboratory-scale forensic bioreactor was built with readily-available, inexpensive materials, and can be easily reproduced for use in forensic research. This research introduces a new technological system, the forensic bioreactor, in order to provide controlled and reproducible environments for forensic science.

DEDICATION

This work is dedicated to my parents Isaiah and Veronica Mealing for their abundant love, encouragement, and support throughout my life and education. They have always kept me motivated and provided me with guidance and thanks to their sacrifices I was fortunate to have this opportunity.

ACKNOWLEDGMENTS

I would like to thank my advisor Dr. Melinda Harman for all of her guidance, support, and patience throughout my graduate experience. Thanks to committee members Dr. Caye Drapcho and Dr. Elena Mikhailova for their support. I would also like to thank Melissa McCullough (Electrical & Communication Services Coordinator, Clemson University Department of Bioengineering), Dr. Daniel Wescott (Director, Texas State University FACTS) and Dr. Kathy Moore (Director, Clemson University Agricultural Service Laboratory) for technical support. Finally I would like to thank the RE-MED laboratory members for their assistance and support. Financial support was provided by the Department of Sociology & Anthropology, the Department of Bioengineering and the Clemson (URGC) Project Initiation Grant.

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CHAPTER ONE

SIMULATING BURIAL SETTINGS: LABORATORY-SCALE FORENSIC BIOREACTOR

INTRODUCTION

Forensic taphonomy involves analysis of evidence specific to environmental factors contributing to postmortem changes on human remains (Haglund, 2006; Pokines, 2013). Variations in environmental conditions represent a substantial challenge to such forensic investigations due to their impact on evidence recovered in an outdoor context. Recognizing this gap, the Scientific Working Group for Forensic Anthropology (SWGANTH) has called for studies involving the use of existing datasets to characterize environmental conditions in diverse climates. A forensic bioreactor that is capable of simulating relevant environmental parameters would broadly impact forensic anthropology through improved understanding of decomposition under controlled environmental conditions.

Bones and soft tissues in varied environmental conditions can experience acceleration or deceleration of the natural decomposition processes, which makes it difficult to interpret data from field studies (White, 1983; Pate, 1989; Marchenko, 2001; Jagers, 2009; Carter, 2010; Howes, 2012). However, soil temperature, soil moisture, and soil chemistry are not easily controlled in a field study.

Using field data accumulated over 20 years, Vass (2011) identified a subset of key environmental factors contributing to varied decomposition, namely temperature and soil conditions (temperature, moisture, pH) for soils typical of the mid to eastern region of the United States. Dunphy et al. (2015) linked relevant parameters identified in forensic

taphonomy to metrics within soil science and discretized key parameters useful for more controlled studies of decomposition. The key parameters were: 1) soil temperature determined from the soil temperature regime for the geographic location of the soil; 2) soil moisture determined from the soil moisture regime for the geographic location of the soil; 3) soil type determined from the soil order; 4) burial depth determined from the horizon in which the soil was collected; and 5) soil pH and soil chemistry as determined from laboratory soil science analysis (Dunphy, 2015). Those parameters proved useful in a simple system capable of simulating a broad range of environmental conditions. However, Dunphy et al. (2015) recognized the need for test chambers that could be controlled and monitored throughout simulation. Such engineered test chambers have the potential to minimize the strong dependency on fieldwork in forensic taphonomy.

Engineered test chambers, or bioreactors, allow for the reproduction and control of specific environments with the goal of understanding biological, chemical, or physical processes. In bioengineering, bioreactors have been designed to manipulate pH, temperature, oxygen content, nutrient supplies to microbial cultures, moisture content, and other parameters (Pörtner, 2005; Sierad, 2010). Bioreactors have rarely been used to analyze stages of decomposition. Previous bioreactors used in decomposition studies failed to simulate key parameters derived from forensic taphonomy and soil science, making it difficult to generalize the findings to global environmental characteristics (Carter, 2008; Abdel-Maksoud, 2010; McLaughlin, 2011).

The purpose of this study was to develop an engineered test chamber, hereafter a forensic bioreactor, to systematically vary key environmental parameters (soil temperature, soil moisture, soil pH) known to impact decomposition. The specific

objectives of this study were to design and build forensic bioreactor, define parameters that are relevant to burial settings and suitable for laboratory simulation in the bioreactor, and verify the performance of the bioreactor.

METHODS AND MATERIALS

Forensic bioreactor design

A forensic bioreactor was designed to improve understanding of bone decomposition under controlled environmental conditions. The forensic bioreactor system consisted of three main components, as shown in Fig. 1 and Fig. 2. The main components of the forensic bioreactor design were: 1) the housing with individual soil chambers, 2) sensors and signal controls, and 3) a computer with software (LabVIEW 2010 service pack 1, National Instruments Corp., Austin, TX) to monitor and control the simulated environmental conditions.

The housing was a modified 0.09 m³ capacity double door compact refrigerator (Model GDE03GGHBB, General Electric, Rapid City, SD). The 0.07 m³ lower compartment housed individual soil chambers and sensors while the 0.03 m³ freezer compartment remained empty throughout experimentation. Eight slots were cut into the door gasket to allow space for the sensor wires to move between the inside of the housing and the outside connection with the signal controller without losing insulation integrity. Therefore, the sealed door of the housing provided for minimal disruption to the temperature and moisture environment maintained within it. The individual soil chambers within the housing were rectangular (21.6 x 17.1 x 6.4 cm) glass containers (pyrex, World Kitchen, LLC, Rosemont, IL) without lids that provided for ease of cleaning and

sterilization and accommodated adequate soil volumes and bone samples required for analysis.

The sensors and signal controls are comprised of three independent temperature sensors (TMP-BTA, Vernier Software, Beaverton, OR), three independent moisture sensors (SMS-BTA, Vernier Software, Beaverton, OR), two digital acquisition boards (DAQ) (290760A, Vernier Software, Beaverton, OR), and a relay control unit (JQX-15F, SparkFun, Niwot, CO). These components function to ensure the system can monitor and control the soil temperature and soil moisture continually while recording the data over 30 days. Key specifications for the temperature sensors include the operating temperature range (-40 – 135 °C) and the accuracy (± 0.2 °C at 0 °C and ± 0.5 °C at 100 °C). Key specifications for the soil moisture sensors include the operating moisture range (0 – 45% volumetric water content (VWC)), resolution (0.05%) and accuracy ($\pm 4\%$ VWC). VWC is considered a measure of soil moisture because it reflects the volume of water per the volume of soil, where volume is the ratio of mass to density. Key specification for the DAQ include 3 analog-13 bits single ended channels and one digital sensor channel, a screw terminal connector including 2 analog input channels-13 bits single ended, 4 digital I/O lines, and one analog output channel, and a maximum sampling rate of up to 48,000 samples per second.

The signal monitors consisted of the two sensor DAQs, one DAQ used to continuously collect the temperature data from the three soil temperature sensors and a second DAQ used to continuously collect the soil moisture data from the three soil moisture sensors. The signal control consisted of a temperature control loop, which connected the temperature sensors' DAQ board to the relay control unit, with

input/output from those devices monitored and controlled by the LabVIEW interface. The relay control unit acted as a switch to regulate the higher voltage needed to operate the refrigerator compressor motor. It was activated by the 0 to 5V general-purpose input/output received from LabVIEW through the temperature sensor DAQ. The relay is controlled through a transistor and connected to a 15 amp, 125 volt GCFI outlet, which allowed the compressor to be safely controlled with lower input/output voltages.

The computer for monitoring and control implemented a LabVIEW program to configure all of the sensors and signal controls. The virtual instrument (VI) block diagram consisted of three main sections (Appendix A), namely temperature data acquisition, soil moisture data acquisition, and the temperature control loop. In the temperature data acquisition section, data were collected from channels 1-3 on the designated temperature sensor DAQ at a specified rate (1 sample/30 minutes). The temperature sensors utilize a variable resistor thermistor that varies non-linearly with temperature, with the measured resistance converted to temperature ($^{\circ}\text{C}$) using the Steinhart-Hart equation, as follows:

$$T = [K_0 + K_1(\ln 1000R) + K_2(\ln 1000R)^3]^{-1} - 273.15 \quad (1)$$

where T is temperature ($^{\circ}\text{C}$), R is the measured resistance in $\text{k}\Omega$, $K_0 = 1.02119 \times 10^{-3}$, $K_1 = 2.22468 \times 10^{-4}$, and $K_2 = 1.33342 \times 10^{-7}$. Similarly, in the soil moisture data acquisition section, data is collected from channels 1-3 on the designated moisture sensor DAQ at a specified rate (1 samples/30 minutes). The moisture sensors use capacitance to measure dielectric permittivity to create a proportional voltage and therefore the VWC of the soil. The temperature data and VWC data are then saved to an Excel file, along with a

time stamp for each data point. Finally, the VI implemented case structures as needed for the temperature control loop described above.

The VI also provided simple user interaction through a screened front panel interface (Fig. 3). This panel allowed the user to specify the DAQs for data collection, to control the measurement frequency, and to set the temperature range of the forensic bioreactor. Specifically, the temperature regime being simulated was maintained by setting high temperature and low temperature limit values that defined the operating boundary condition for the temperature control loop. Those settings triggered the refrigerator compressor to switch on and off at those range limits throughout the experimental run.

Parameters relevant to burial settings

The forensic bioreactor was designed to allow control of key environmental parameters relevant to burial settings, namely soil type, burial depth, soil pH, soil temperature, and soil moisture. Specific ranges of these parameters were defined to make them comparable with sensor outputs and useful for laboratory simulation in the forensic bioreactor.

Two relevant soil media (Ultisol, SC and Mollisol, TX) and one control media were acquired. All soils were collected from 50 cm depth to mimic soil conditions that would exist in a shallow grave (Fig. 4). Ultisol, characterized by low pH, was obtained at the Simpson Agricultural Station in Pendleton, SC (Ismail, 1993). This soil was from a Hiwassee sandy loam having a 10 to 15% slope (Fine, kaolinitic, thermic Rhodic Kanhapludults). A kaolinitic soil consists of layered silicate mineral clay. Due to the

relative unavailability of non-acidic soils in the southeast United States, Mollisol was obtained from Texas State University Forensic Anthropology Center in San Marcos, TX. This soil had a neutral pH and was from a Comfort-Rock outcrop complex having a 1 to 8% slope (Clayey-skeletal, mixed, thermic Lithic Argiustolls). As a control media, 2.0 mm diameter glass beads (Propper Manufacturing Co, Long Island City, NY) were used as a suitable control because of their inert surface chemistry and similarity in size relative to the soil particulate (Fig. 5). After collecting the soils and completing an initial laboratory soil science analysis (Table 1), it was determined that both soils have a thermic temperature regime (between 15° – 22° C). Ultisol commonly has udic moisture regime. Mollisol in Texas had ustic moisture regime, but since Mollisol can have udic moisture regime as well, udic moisture regime was chosen for this study. A udic moisture regime was simulated using a VWC above 10%, which is a noted baseline moisture level of “moist” soils (Mount, 2002). Thus, the thermic temperature and udic moisture regime were the parameters simulated in the forensic bioreactor.

Forensic bioreactor performance verification

Four experiments were designed to verify that the forensic bioreactor could provide for independent monitoring and recording of all sensors, achieve the specified parameters to simulate environmental conditions, and control and maintain those parameters for sensors in different soil chambers. The simulated environmental conditions included the two soil types described in the previous section, a temperature range of 18° C – 20° C and a VWC of > 10%, which are consistent with a thermic temperature regime and a udic moisture regime, respectively.

The first experiment was to verify the temperature sensors could independently detect simulated temperature conditions and provide feedback to the temperature control loop. The temperature sensors were exposed to three different water baths ranging in temperature from 0 °C to 40 °C. The sensors were monitored by the temperature control loop, which activated the refrigerator compressor as the temperature rose above the upper limit of 20 °C and deactivated the compressor when the temperature fell below the lower limit of 18°C, as specified by the user. As the LabVIEW VI ran, all three temperature sensors were simultaneously placed in the hot (40° C) water bath for 30 seconds, then transferred to a cold (0° C) water bath for 30 seconds, then finally transferred to an ambient (21° C) water bath. This experiment was repeated in three trials.

The second experiment was to verify the ability of the temperature control loop to monitor the temperature sensors and control the refrigerator compressor in order to maintain the set temperature parameters within a stable range for an extended time period. Three temperature sensors were placed in one chamber of the control media (2.0 mm diameter glass beads). Temperature data were recorded with the upper temperature limit set to 20° C and the lower temperature limit set to 18° C. Temperature data were recorded every 10 minutes over 70 hours.

The third experiment was to verify the moisture sensors could independently detect changes in VWC. Soil moisture was altered by incrementally adding different volumes of water to the two types of soil media (Ultisol and Mollisol) and the control media. Each soil was uniformly sized using a standard 12.7 mm sieve and then water was added and thoroughly mixed into the soil. The moisture sensors were buried at a 3.8 cm depth in individual soil chambers containing a given soil medium and then the soil was

compressed with a flat plate and an impact load applied by dropping a 2.3 kg barbell from a 1 meter height. This ensured homogenous compaction for each soil medium. The sensor measurement of VWC was collected and this process was repeated three times so that each sensor was verified in each media.

The fourth experiment was to verify the moisture sensors could monitor soil VWC and that soil moisture was maintained over an extended time period. Moisture sensors were individually placed in the two types of soil media (Ultisol and Mollisol) and the control media. Soils were sieved, moistened by adding 250 mL of water, mixed and compacted as described previously. Moisture data were recorded every 10 minutes over 70 hours.

RESULTS AND DISCUSISON

Bioreactor design

The forensic bioreactor was developed and assembled at the Clemson University Biomedical Engineering Innovation Campus. The laboratory-scale forensic bioreactor was built with readily-available, inexpensive materials, and can be easily reproduced for use in forensic research. The main components of the forensic bioreactor provided for temperature control relevant to specific soil temperature regimes known to impact bone decomposition, moisture monitoring relevant to specific soil moisture regimes known to impact bone decomposition, application of temperature and moisture sensors, ease of monitoring, durability for extended use, and ease of placement of soil and bone samples.

The design of the forensic bioreactor was informed by simplifying assumptions about key environmental parameters relevant to forensic taphonomy. The forensic

bioreactor was capable of simulating burial settings through the use of two soil types with different soil pH levels along with sensors and signal controls to maintain a thermic temperature regime (15 – 22° C) and a udic moisture regime (>10% VWC). Through various experiments described in subsequent sections, this forensic bioreactor was proven capable of systematically controlling and monitoring key environmental parameters known to impact bone decomposition.

Temperature sensor and temperature control loop verification

The temperature sensors independently detected the simulated temperature conditions and provided feedback to the temperature control loop. The relay control unit switched on and allowed current flow to activate the forensic bioreactor's cooling system (refrigerator compressor) when the temperature exceeded the pre-set upper temperature limit and switched off to inactivate the compressor when the temperature fell below the lower temperature limit (Fig. 6). All temperature sensors collected independent measurements and the relay control unit responded to these measurements based on the bounds set in the case structures.

The temperature control loop successfully monitored the temperature sensors and controlled the refrigerator compressor over the course of 70 hours. During that timeframe, the compressor switched on 13 times and was activated a total of 10.8 hours over the entire duration (Fig. 7). The temperature stayed within the set range of 18° C and 20° C. Rapid cooling, as evidence by the sharp decline of the recorded temperatures once the upper temperature limit was reached, indicates the temperature control loop functioned within the programmed case structures by triggering the relay to turn on the

refrigerator compressor. Once the sensors detecting the lower temperature limit triggered off the compressor, the temperature range was maintained over approximately 4.5 hours without the need for additional cooling by the compressor. These data indicate that the closed system (sealed door on the insulated refrigerator unit) provided temperature control and maintenance, with infrequent operation of the compressor.

It should be noted that although the forensic bioreactor temperature parameters remained within the thermic temperature regime (15 – 22° C), the range toggled between 18.8° C and 20.2° C. The LabVIEW code truncated the temperature sensor outputs and thus the compressor was deactivated when the average temperature sensor output was <19° C. Adjusting the case structure specifications to include tenth degrees of measured values will allow the system to toggle more closely to the temperature range set by the user in the LabVIEW interface.

Moisture sensor verification

The soil moisture sensors independently measured the simulated soil moisture conditions and detected changes in VWC. All three moisture sensors detected an increase in VWC with increasing added volumes of water (Fig. 8). The soil moisture sensors monitored soil VWC, which demonstrated the forensic bioreactor maintained a VWC within the udic soil moisture regime (>10%) over the course of 70 hours (Fig. 9). The VWC averaged 22.0%, 17.6%, and 23.2% in the control, Ultisol, and Mollisol media, respectively. The soil moisture parameters declined slowly, decreasing an average of 0.9% over the course of this experiment. Linear regression analysis indicates there was significant water loss (Fig. 9), as the slopes of VWC% versus time plot were significantly

different from 0 (t-test, $p < 0.001$). Longer duration experimental runs would require a system to add moisture to the soils, as VWC ultimately would drop below 10%.

CONCLUSIONS

The purpose of this study was to develop an engineered test chamber, hereafter a forensic bioreactor, to systematically vary key environmental parameters (soil temperature, soil moisture, soil pH) known to impact decomposition. This research introduced a new technological system, the forensic bioreactor, in order to provide controlled and reproducible environments for forensic science. The forensic bioreactor provided for independent monitoring and recording of the temperature sensors and moisture sensors, and achieved the pre-defined parameters to simulate the relevant environmental conditions. In future studies, the forensic bioreactor is intended for use in assessing changes in bone and soil properties during decomposition as related to the key environmental parameters.

APPENDICES

Appendix A

Figures



Fig. 1 – The main components of the forensic bioreactor design included the housing, internal sensors, external signal controls, and a computer with software to monitor/control the desired environmental conditions.



Fig 2. – Inside the housing there were: (a) individual soil chambers with sensors; (b) a gasket seal through which the sensor wires were passed. External to the housing were: (c) the signal controls including DAQs boards and relay control unit, and (d) the computer and software providing a user interface.

SET TEMPERATURE RANGE

SensorDAQ Device 2

1%

Upper Limit (C) 3

0

Lower Limit (C) 2

0

Channel Name: 3 /ao0

SENSOR DATA

Average

-292.15

Temp 1

-292.15

Temp 2

-292.15

Temp 3

-292.15

Soil Moisture 1

-42

Soil Moisture 2

-42

Soil Moisture 3

-42

Sample Rate (Hz)

10

Samples to Read

50

STOP

CONFIGURE FIRST SENSORDAQ

Sensor Port

0

Data Channel

5V

SensorDAQ Device

1%

Channel Name: /_sensor0_5V

Sensor Port

0

Data Channel 3

5V

SensorDAQ Device 3

1%

Sensor Port 4

0

Data Channel 4

5V

SensorDAQ Device 4

1%

CONFIGURE SECOND SENSORDAQ

Sensor Port 2

0

Data Channel 2

5V

SensorDAQ Device 2

1%

Channel Name: /_sensor0_5V

Sensor Port 5

0

Data Channel 5

5V

SensorDAQ Device 5

1%

Sensor Port 6

0

Data Channel 6

5V

SensorDAQ Device 6

1%

Fig. 3 – Screenshot of the front panel user interface from the LabVIEW VI.

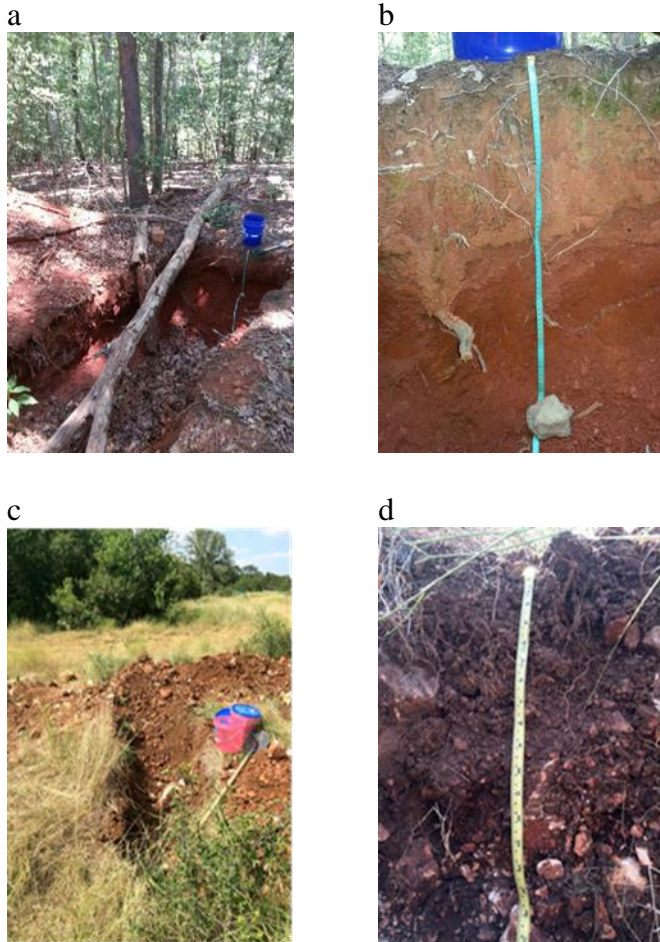


Fig. 4 – Collection sites for the two soil media: (a) Sampling site at Simpson Agricultural Station site in Pendleton, SC: (b) Soil profile for Hiwassee sandy loam, 10 to 15% slope (Fine, kaolinitic, thermic Rhodic Kanhapludults), Map unit symbol: HaD; (c) Sampling site at the Texas State Forensic Anthropology Center in San Marcos, TX: (d) Soil profile for Comfort-Rock outcrop complex, 1 to 8% slope (Clayey-skeletal, mixed, thermic Lithic Argiustolls). Map unit symbol: CrD.

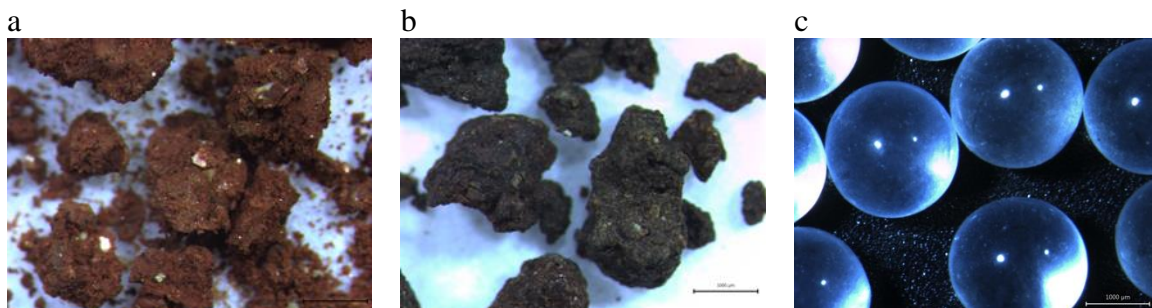


Fig. 5 – Digital images of the soil media: (a) Ultisol, (b) Mollisol, (c) control (glass beads). Images were acquired using a reflective light optical microscope at 12X magnification. The scale bar at bottom right of each image represents 1000 μm .

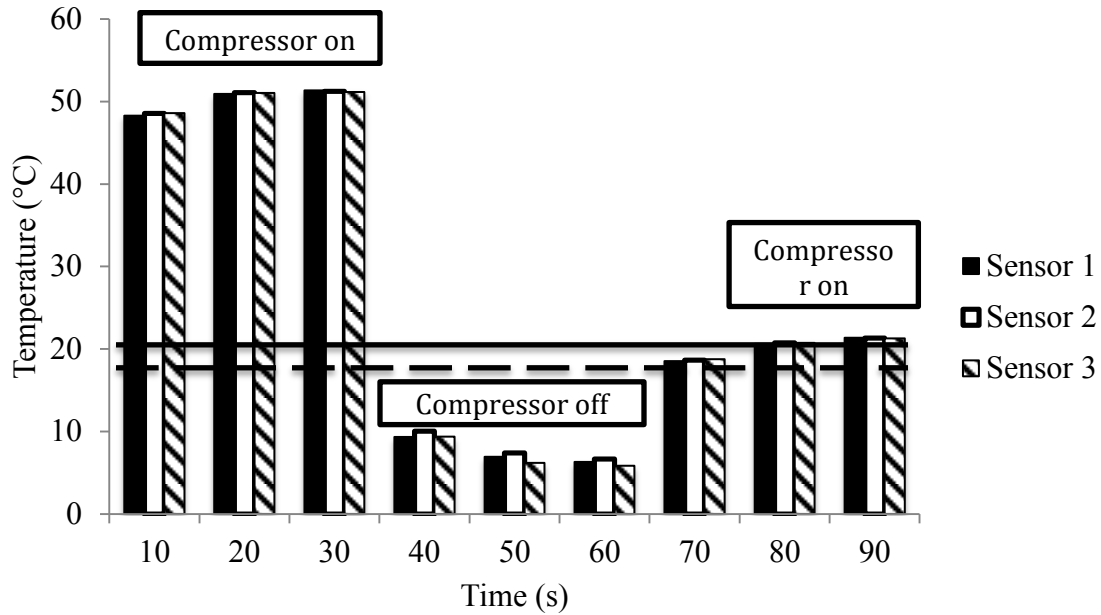


Fig. 6 – Verification of the temperature sensors to independently detect simulated temperature conditions and provide feedback to the temperature control loop. Recorded data included the temperature output of the three temperature sensors placed in water baths with different temperatures. The solid and dashed horizontal lines indicate the upper and lower temperature limits set for the temperature control loop in LabVIEW, respectively. From time 0 – 30 seconds, sensors in the 40° C bath triggered the relay to turn on the refrigerator compressor because the sensor value monitored by the temperature control loop exceeded the 20° C upper limit. From time 40-60 seconds, sensors in the 0° C bath triggered the relay to turn off the refrigerator compressor because the sensor value was below the 18° C lower limit. From time 80 – 90 seconds, sensors in the 21° C bath did not trigger the relay to turn on the refrigerator compressor until the sensor value exceeded the upper limit. Each bar represents the average of 3 trials.

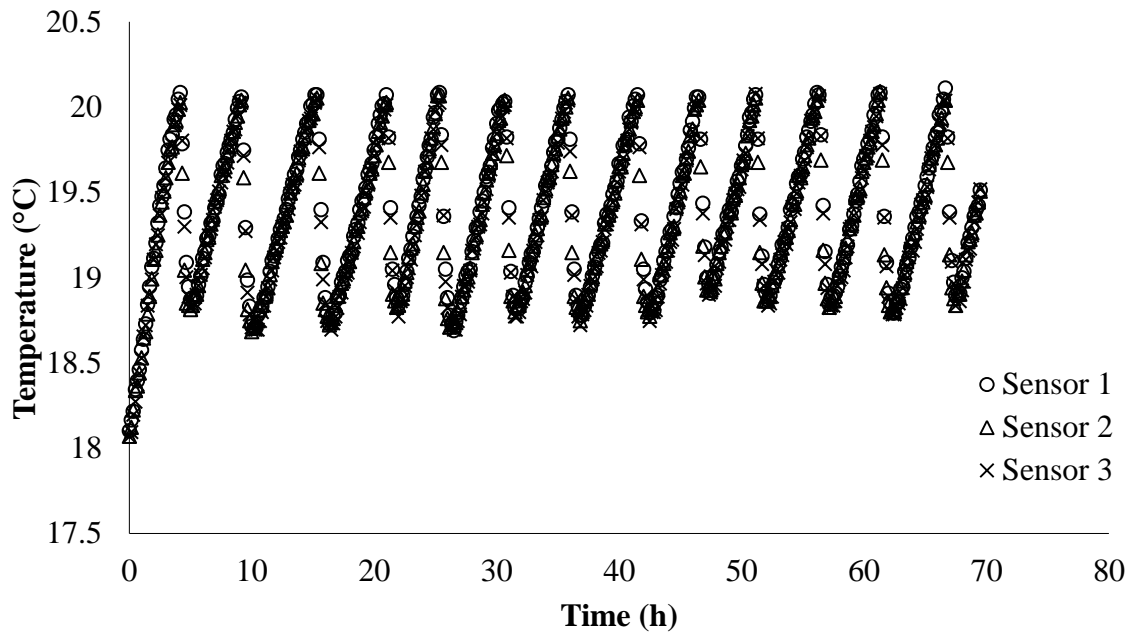


Fig. 7 – Verification of the temperature control loop to function within the programmed case structures by monitoring the temperature sensors and controlling the refrigerator compressor.

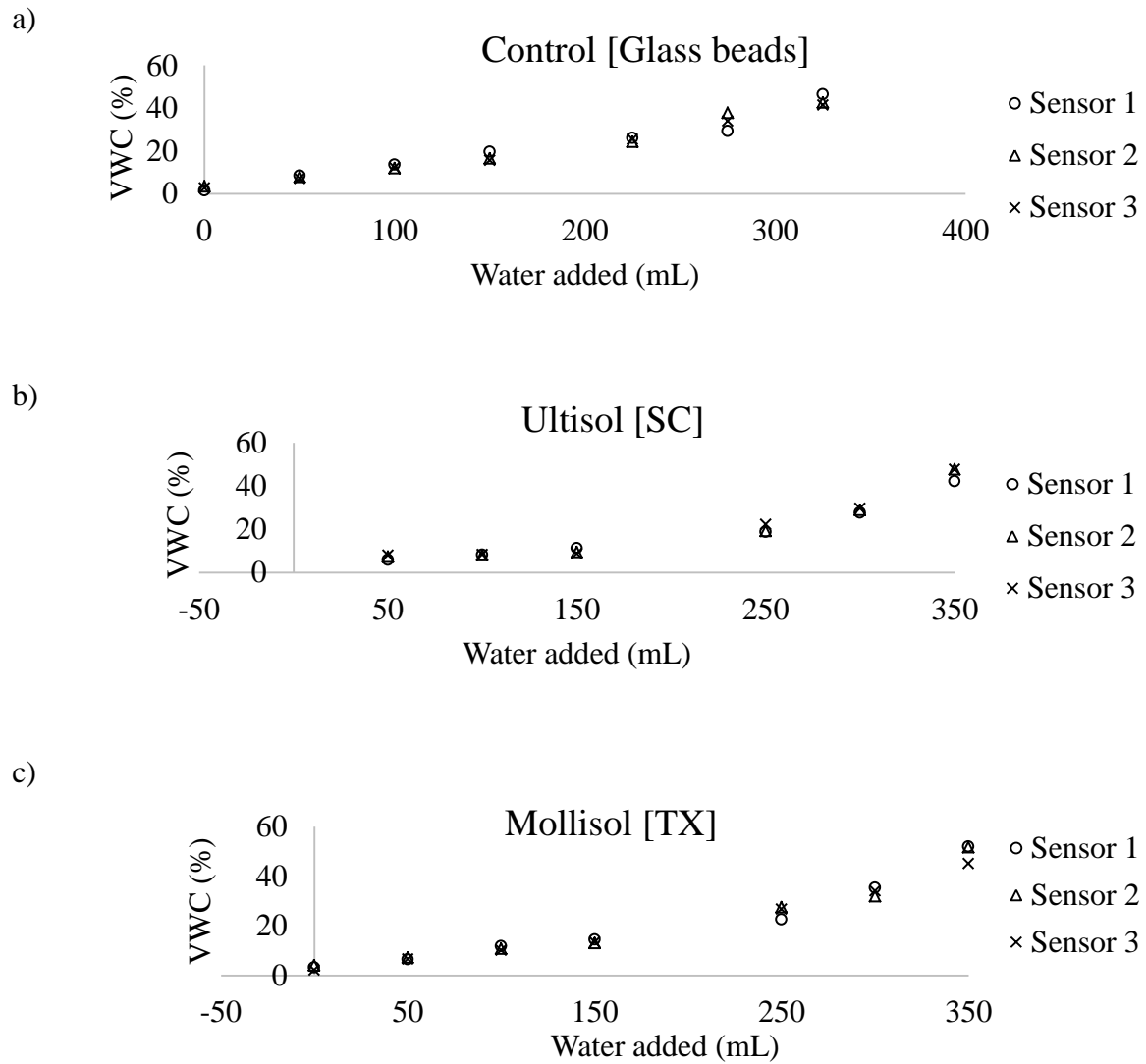


Fig. 8 – Verification of soil moisture sensors to independently measure changes in VWC in: (a) control (glass beads), (b) Ultisol, and (c) Mollisol.

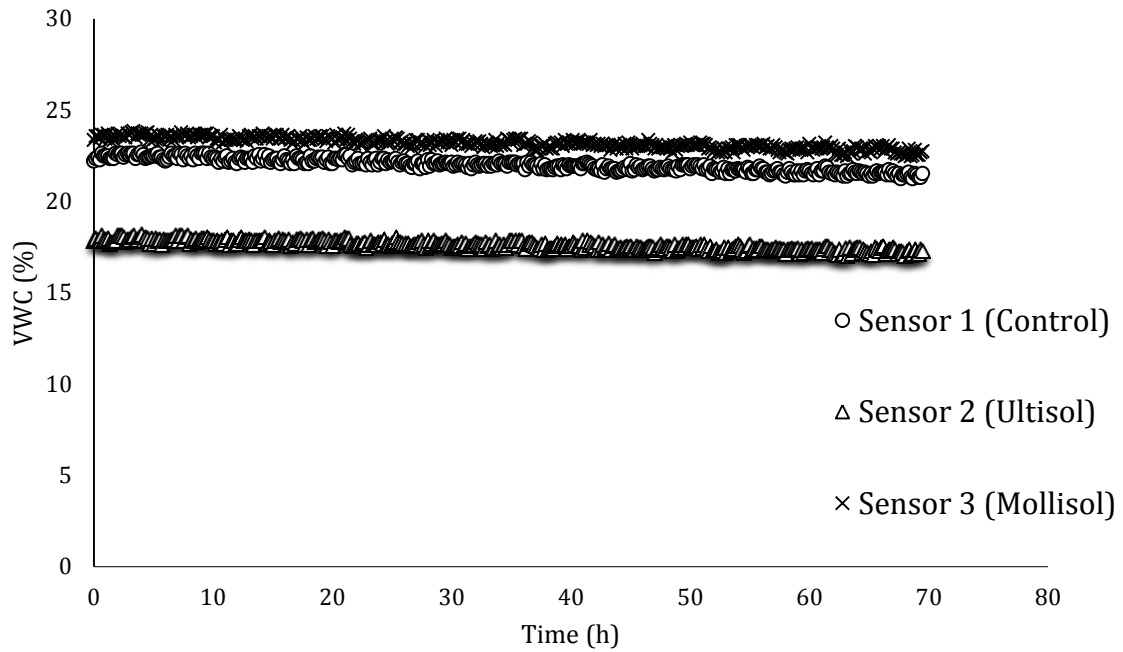


Fig. 9 – Verification of the moisture sensors to monitor soil VWC in two types of soil media (Ultisol and Mollisol) and the control media over 70 hours. The slope of each regression line indicates that the VWC decreased at a rate of -0.015 %VWC/hour, -0.010 VWC%/hour and 0.013 VWC%/hour for the control, Ultisol, and Mollisol media, respectively.

Appendix B

Tables

Table 1 – Soil parameters for the two soil media obtained from the laboratory soil science analysis (CUAGSL_1, 2016; CUAGSL_2, 2016; TXAM 2016).

Soil data	Sampling Site Data	
	Hiwassee sandy loam, 10 to 15 % slope (Pendleton, SC)	Comfort-Rock outcrop complex 1 to 8 % slope (San Marcos, TX)
Soil order	Ultisol	Mollisol
Soil moisture regime	Udic	Ustic ^d
Soil temperature regime	Thermic (15-22°C)	Thermic (15-22°C)
Sand, %	36	47
Silt, %	19	12
Clay, %	45	41
Soil texture class	Clay	Clay
pH	5.5	7.0
C, %	3.5	2
P, mg/kg	0.5	10
K, mg/kg	25.5	218
Mg, mg/kg	61.5	176
Ca, mg/kg	41	4968
Zn, mg/kg	0.35	0.40
Mn, mg/kg	1	39.46
Cu, mg/kg	0.35	0.77
B, mg/kg	0.2	0.38
Na, mg/kg	5.5	6
CEC, ^a meq/100g	4	N/A
BS, ^b %	20	N/A
OM, ^c %	10.6	3.6

^a Cation exchange capacity

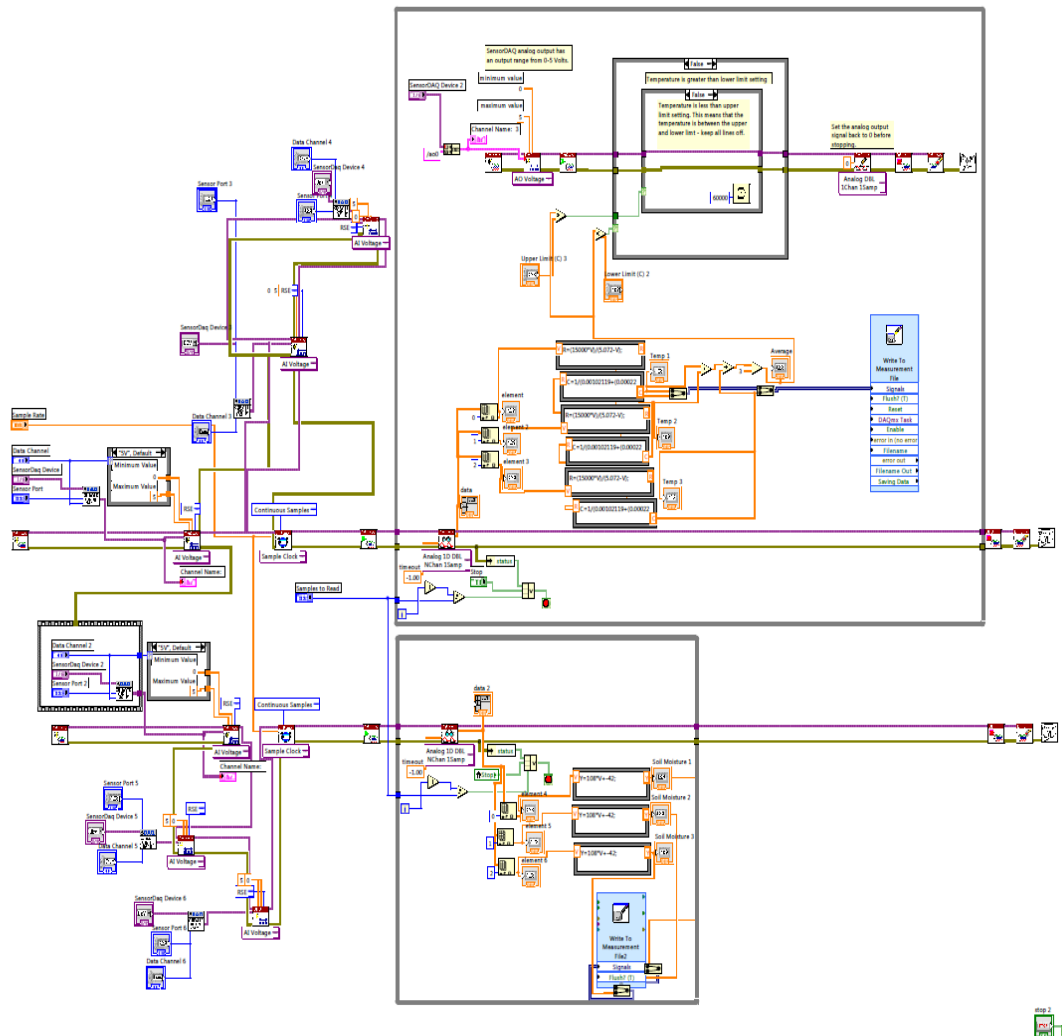
^b Base saturation

^c Organic matter

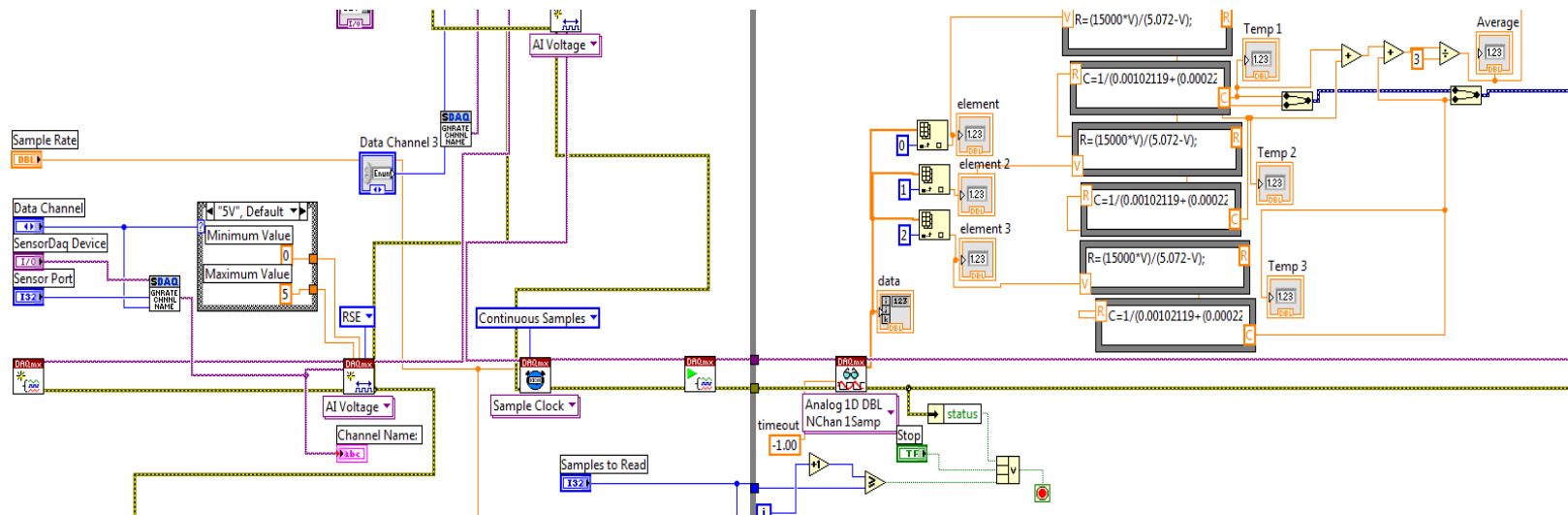
^d Note: Ultisol has udic moisture regime. Mollisol in TX has ustic moisture regime as indicated in the taxonomic name, but Mollisol also can be found with udic moisture regime.

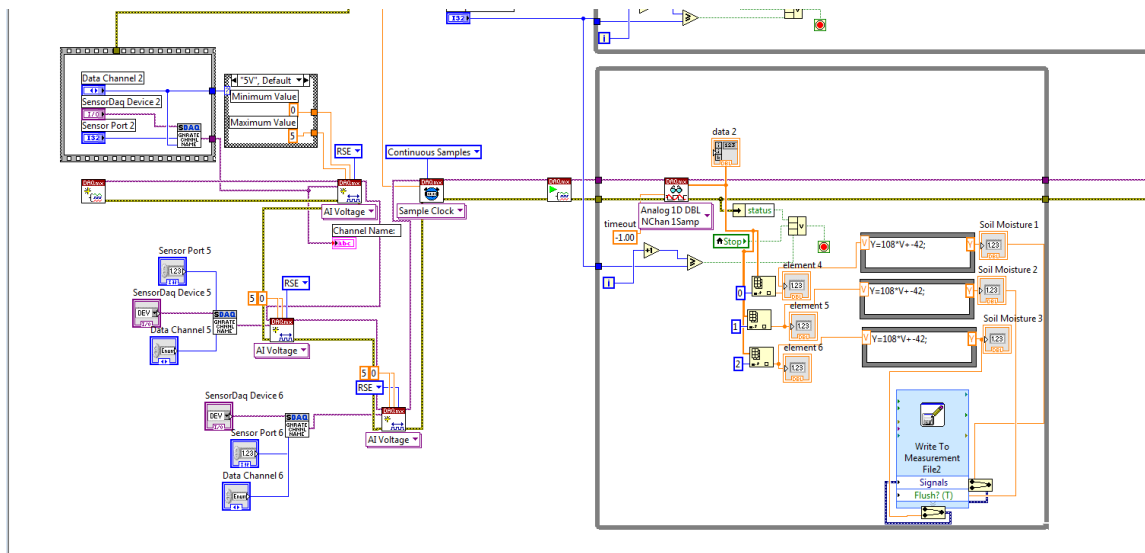
Appendix C

LabVIEW Virtual Instrument Block Diagram

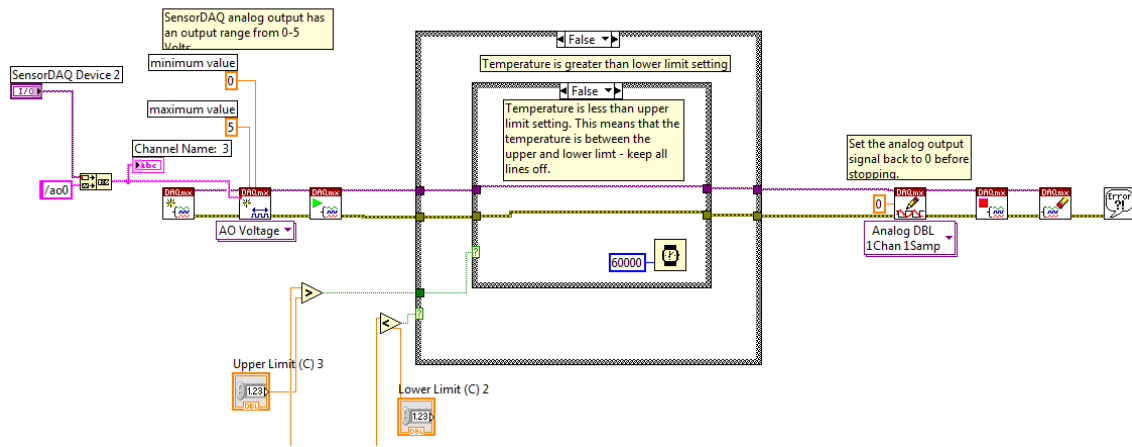


Appendix C.1: Full block diagram of V.





Appendix C.3: Soil moisture data acquisition portion of block diagram.



Appendix C.4: Temperature control unit portion of block diagram.

Appendix D

Soil Analysis Methods

Appendix D.3 Soil lab methods (Ultisol):

Sample Extraction Procedure (Mehlich 1)

After samples are checked for proper order, labeled with Check and Blank locations noted, samples are ready for extraction.

- 1 Using a 4 mL volumetric scoop (assume 5 g), measure an amount of soil from each sample box using the following method:
 - Dip scoop with sweeping motion and fill to overflowing
 - Hold scoop over box and firmly tap handle three times to settle
 - Strike off excess soil with leveling rod and transfer
- 2 Measure samples into extraction racks containing 10 polyethylene cups each. A Check sample is scooped at the appropriate location from a separate Check sample box or no sample is scooped at the blank location.
- 3 Extract fifty samples at a time. Add twenty milliliters of Mehlich 1 extracting solution ($0.05\text{N HCl} + 0.025\text{N H}_2\text{SO}_4$) by automatic pipette to each sample.
- 4 Shake samples on a mechanical reciprocating shaker, adjusted to 180 oscillations per minute with a 4 cm stroke, for 5 minutes.
- 5 Place prefolded, high quality filter paper, moistened with deionized water into funnel tubes in racks that correspond with the extraction racks.

- 6 After shaking, immediately filter and save the collected extract for mineral analysis (P, K, Ca, Mg, Na, Zn, Mn, Cu, B). Transfer to test tubes for ICP.
- 7 All glassware and cups should be thoroughly rinsed between samples with deionized water. Weekly wash glassware using a minimum of detergent and rinse thoroughly.

Mehlich 1 Extracting Solution (0.05 N HCl + 0.025 N H₂SO₄)

To prepare 18 liters: add 77 mL concentrated HCl and 13 mL concentrated H₂SO₄ to approximately 15 liters of deionized water in 20 liter carboy. Bring to 18 liters with deionized water and mix thoroughly.

Appendix D.4: Soil analysis methods (Mollisol)

Preparation of soil samples

All soil samples should be removed from their initial shipping containers, placed in aluminum or other non-porous, non-corrodible shallow containers and oven dried at 65C (plus or minus 2C) in a forced air oven for 16 hours or until dry. Following oven drying, samples are pulverized using a open mesh bottom hammer style soil pulverizer (as a reference only, common manufacturers of these systems include Agvise, Dynacrusher and Humboldt). All soil exiting the pulverizer is screened to remove all particles greater than 2mm. It is vital that the pulverization step is not overly aggressive and break down small rocks or individual soil separates. The use of disk mills, ring and puck mills, mortar and pestles, cone crushers or other fixed opening mills are not appropriate for use when Texas A&M AgriLife Extension Service soil fertility recommendations are utilized.

Mehlich III (Phosphorus and multi-nutrient extractant)

Phosphorus, K, Ca, Mg, Na and S are extracted using the Mehlich III extractant and are determined by ICP. The extractant is a dilute acid-fluoride-EDTA solution of pH 2.5 that consists of 0.2 N CH₃-COOH-0.25 N NH₄NO₃-0.015 N NH₄F-0.013 N HNO₃-0.001 M EDTA. The method estimates plant available pools of the elements listed above and is currently the only method recognized by Texas AgriLife Extension Service. Reported on a dry soil basis only.

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Soil pH (referred to as soil water pH)

Soil pH is determined in a 1:2 soil:water extract of the soil using deionized water.

Samples are stirred and allowed to equilibrate for a minimum of 30 minutes after adding the water. The actual determination is made using a hydrogen selective electrode.

Reported on a dry soil basis only.

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